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Molecular docking studies of some new imidazole derivatives for antimicrobial properties

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Abstract   In modern drug designing, molecular docking is routinely used for understanding drug-receptor interaction. In the present study six imidazole derivatives containing substituted pyrazole moiety (**2a,b** and **4a–d**) were synthesized. Structures of the newly synthesized compounds were characterized by spectral studies. Compounds were screened for their antibacterial activity. Compound **4c** was found to be potent antimicrobial against *Pseudomonas aeruginosa* at concentrations of 1 and 0.5 mg/mL compared to standard drug Streptomycin. All the compounds were subjected to molecular docking studies for the inhibition of the enzyme L-glutamine: D-fructose-6-phosphate amidotransferase [GlcN-6-P] (EC 2.6.1.16). The *in silico* molecular docking study results showed that, all the synthesized compounds having minimum binding energy and have good affinity toward the active pocket, thus, they may be considered as good inhibitor of GlcN-6-P synthase.

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1. Introduction

Molecular docking may be defined as an optimization problem, which would describe the "best-fit" orientation of a ligand that binds to a particular protein of interest and is used to predict the structure of the intermolecular complex formed between two or more molecules. The most interesting case is the protein ligand interaction, because of its applications in medicines. Ligand is a small molecule, which interacts with protein's binding sites. There are several possible mutual conformations in which binding may occur. These are commonly called binding modes (Sharma et al., 2010). In modern drug

designing, molecular docking is routinely used for understanding drug-receptor interaction. Molecular docking provides useful information about drug receptor interactions and is frequently used to predict the binding orientation of small molecule drug candidates to their protein targets in order to predict the affinity and activity of the small molecule.

Human beings have been in constant exposure to pathogens for many decades. Invasive microbial infections are major problems around the world, especially in immuno compromised patients. The recent expansion of antimicrobial drug research has occurred because there is a critical need for new antimicrobial agents to treat these life threatening invasive infections. The development of antimicrobial resistance has increased in this century and there is a need for developing new antimicrobial agents which will be more selective, potent and less toxic compared to the existing drugs in clinical treatment. Heterocycles containing an azole ring system are found to exhibit a wide spectrum of biological activities, including antibacterial and antifungal properties. Imidazole and its derivatives have gained remarkable importance due to their widespread biological activities and their use in synthetic chemistry. Imidazole derivatives possess a broad spectrum of pharmacological activities such as, anti-inflammatory (Suzuki et al., 1992), analgesic, anti-convulsant (Pinza et al., 1993), antitubercular (Pandey et al., 2009), antimicrobial, anticancer and anti-Parkinson (Miyachi et al., 1998) activities. Imidazole and its derivatives are of great significance due to their important roles in biological systems, particularly in, enzymes as proton donors and/or acceptors, coordination system ligands and the base of charge–transfer processes. The imidazole nucleus appears in a number of naturally occurring products like, amino acids histidine and purines, which comprise many of the most important bases in nucleic acids.

Similarly pyrazole derivatives have showed significant biological activities, such as anti-microbial (Isloor et al., 2009), analgesic (Isloor et al., 2000), anti-inflammatory (Bekhita and Abdel-Aziem, 2004) and anticancer (Dhanya et al., 2009) activities. This gave a great impetus to the search for potential pharmacologically active drugs carrying pyrazole substituents.

The enzyme, namely glucosamine-6-phosphate synthase (GlmS, GlcN-6-P synthase, L-glutamine:D-fructose-6P amidotransferase, EC 2.6.1.16) also known under the trivial name of glucosamine-6-phosphate synthase, is a new target for antifungals (Chmara et al., 1984). GlcN-6-P synthase catalyzes the first step in hexosamine metabolism, converting fructose 6-phosphate (Fru6P) into glucosamine 6-phosphate (GlcN6P) in the presence of glutamine. The reaction catalyzed by GlmS is irreversible, and is therefore considered as a committed step. The end product of the pathway, N-acetyl glucosamine, is an essential building block of bacterial and fungal cell walls. Structural differences between prokaryotic and human enzymes may be exploited to design specific inhibitors, which may serve as prototypes of anti-fungal and anti-bacterial drugs (Borowski, 2000).

It has been shown that even a short time inactivation of GlcN-6-P synthase is lethal for fungal cells, while in mammals depletion of the amino sugar pool for a short time is not lethal, because of the much longer lifespan of mammalian cells, long half lifetime of GlcN-6-P synthase, and rapid expression of the mammalian gene encoding the enzyme GlcN-6-P synthase (Milewski et al., 1986). It is well established that small modifications in the structure of the targets are altering their biological character as well as their physiochemical properties. A

detailed literature survey on antimicrobial activity of various types of compounds indicated that, the presence of certain pharmacophore such as imidazole/pyrazole in any molecule plays an important role in enhancing activity. In our previous paper (Vijesh et al., 2011), we reported the synthesis and the antimicrobial activity of the imidazole derivatives containing pyrazole nucleus. In continuation of that, we have performed the molecular docking studies of the biologically active six compounds for better understanding of the drug-receptor interaction.

2. Experimental

2.1. Materials and methods

Melting points were determined by open capillary method and were uncorrected. The IR spectra (in KBr pellets) were recorded on a JASCO FT/IR-4100 spectrophotometer. ^1H NMR and ^{13}C NMR spectra were recorded (DMSO-d_6) on a Bruker (400 MHz) using TMS as the internal standard. Chemical shift values are given in δ (ppm) scales. The mass spectra were recorded on a JEOL JMS-D 300 spectrometer operating at 70 eV. Elemental analyses were performed on a Flash EA 1112 series CHNS-O Analyzer. The completion of the reaction was checked by thin layer chromatography (TLC) on silica gel coated aluminum sheets (silica gel 60 F254) obtained from Merck. Commercial grade solvents and reagents were used without further purification (see Scheme 1).

2.2. General procedure for the synthesis of new derivatives of 2,4,5-trisubstituted imidazoles (2a,b)

A mixture of 3-aryl-1H-pyrazole-4-carbaldehyde **1a,b** (0.01 mol), benzil (0.01 mol) and ammonium acetate (0.05 mol) in acetic acid (50 mL) was refluxed for 6–7 h at 120 °C. After completion of the reaction, the reaction mixture was allowed to cool and filtered to remove any precipitate. 300 mL of ice-water was added to the filtrate and the precipitated product was collected by filtration. The crude product was recrystallized using ethanol–DMF mixture (Vijesh et al., 2011).
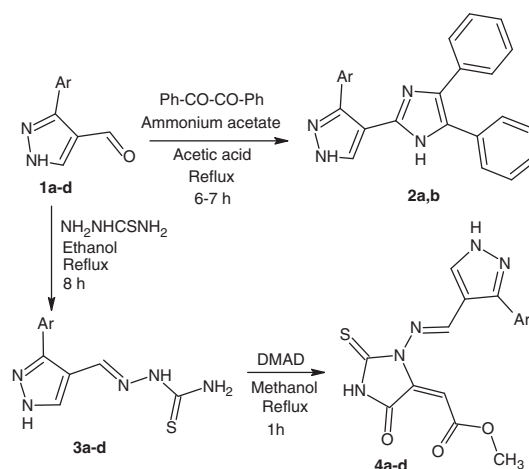
Ar = 4-Thioanisyl, 2,4-Dichlorophenyl, 2,5-Dichlorothiophene, 4-Tolyl

Scheme 1 Synthetic route for the compounds **2a,b** and **4a–d**.

2.2.1. Characterization of synthesized compounds

2.2.1.1. 3-(2,4-Dichlorophenyl)-4-(4,5-diphenyl-1H-imidazol-2-yl)-1H-pyrazole (2a).
IR (KBr, v_{max} cm^{-1}): 3135 (N–H-str), 3062, 2922 (C–H-str), 1668 (C=N), 1598 (C=C); ^{1}H NMR (400 MHz, DMSO-d_6): δ 7.14–7.92 (m, 13H, Ar-H), 8.27 (s, 1H, pyrazole-5H), 12.22 (s, 1H, pyrazole–NH), 13.24 (s, 1H, imidazole–NH); ^{13}C NMR: 194.77, 184.39, 171.94, 140.16, 135.4, 134.5, 133.95, 133.27, 132.22, 131.38, 129.55, 129.46, 128.51, 128.05, 126.55, 120.97, 111.56; MS: m/z = 431.2 (M$^+$), 433.1 (M + 2), 435.1 (M + 4); Anal. calcd. for $C_{24}H_{16}Cl_2N_4$: C, 66.77; H, 3.71; N, 12.98; Found: C, 66.76; H, 3.68; N, 12.95%.

2.2.1.2. 4-(4,5-Diphenyl-1H-imidazol-2-yl)-3-(4-methylphenyl)-1H-pyrazole (2b).
IR (KBr, v_{max} cm^{-1}): 3130 (N–H-str), 3054, 2919 (C–H-str), 1654 (C=N), 1604 (C=C); ^{1}H NMR (400 MHz, DMSO-d_6): δ 2.07 (s, 3H, CH$_3$), 7.16–7.91 (m, 14H, Ar-H), 7.92 (s, 1H, pyrazole-5H), 12.28 (s, 1H, pyrazole–NH), 13.10 (s, 1H, imidazole–NH); ^{13}C NMR: 184.70, 140.62, 137.21, 129.53, 129.44, 128.54, 128.11, 127.91, 127.03, 109.39, 20.98; MS: m/z = 377.2 (M + 1); Anal. calcd. for $C_{25}H_{20}N_4$: C, 79.69; H, 5.31; N, 14.88; Found: C, 79.66; H, 5.28; N, 14.55%.

2.3. General procedure for the synthesis of methyl(2Z)-[3-({(E)-[3-aryl-1H-pyrazol-4-yl]methylidene}amino)-5-oxo-2-thioxoimidazolidin-4-ylidene]ethanoate (4a–d)

An equimolar mixture of 3-aryl-1H-pyrazole-4-carbaldehyde thiosemicarbazone 3a–d (0.01 mol) and dimethylacetylenedicarboxylate (DMAD) (0.01 mol) in methanol (20 mL) was refluxed for 1 h. After completion of the reaction, the reaction mixture was allowed to cool. The solid thus separated was collected by filtration and recrystallized using ethanol–DMF mixture (Vijesh et al., 2011).

2.3.1. Characterization of synthesized compounds

2.3.1.1. Methyl(2Z)-[3-({(E)-[3-(2,4-dichlorophenyl)-1H-pyrazol-4-yl]methylidene}amino)-5-oxo-2-thioxoimidazolidin-4-ylidene]ethanoate (4a).
IR (KBr, v_{max} cm^{-1}): 3242 (N–H-str), 3068, 2951 (C–H-str), 1707 (C=O ester), 1645 (C=O cyclic amide), 1607 (C=N), 1106 (C=S), 1238, 1195 (C-O ester); ^{1}H NMR (400 MHz, DMSO-d_6): δ 3.80 (s, 3H, OCH$_3$), 6.58 (s, 1H, C=CH), 7.41–7.57 (m, 3H, Ar-H), 7.96 (s, 1H, pyrazole-5H), 8.28 (s, 1H, N=CH), 12.64 (s, 1H, pyrazole–NH), 13.50 (s, 1H, imidazole–NH); MS: m/z = 423.9 (M$^+$), 425.9 (M + 2), 427.9 (M + 4); Anal. calcd. for $C_{16}H_{11}Cl_2N_5O_3S$: C, 45.26; H, 2.59; N, 16.50; Found: C, 45.23; H, 2.57; N, 16.47%.

2.3.1.2. Methyl(2Z)-[3-({(E)-[3-(2,5-dichlorothiophen-3-yl)-1H-pyrazol-4-yl]methylidene} amino)-5-oxo-2-thioxoimidazoli-
din-4-ylidene]ethanoate (4b). IR (KBr, v_{max} cm^{-1}): 3213 (N–H-str), 3051, 2953 (C–H-str), 1713 (C=O ester), 1640 (C=O cyclic amide), 1602 (C=N), 1023 (C=S), 1247, 1197 (C–O ester); ^{1}H NMR (400 MHz, DMSO-d_6): δ 3.79 (s, 3H, OCH$_3$), 6.62 (s, 1H, C=CH), 7.23 (s, 1H, Ar-H), 8.35 (s, 1H, pyrazole-5H), 8.38 (s, 1H, N=CH), 12.71 (s, 1H, pyrazole–NH), 13.60 (s, 1H, imidazole–NH); ^{13}C NMR: 165.77, 165.65, 151.60, 142.92, 131.66, 128.72, 125.06, 114.89, 114.13, 52.38; MS: m/z = 430.0 (M$^+$), 432.0 (M + 2). 434.0 (M + 4); Anal. calcd. for $C_{14}H_9Cl_2N_5O_3S_2$: C, 39.04; H, 2.09; N, 16.27; Found: C, 39.03; H, 2.06; N, 16.26%.

2.3.1.3. Methyl(2Z)-(3-{[(E)-{3-[4-(methylsulfanyl)phenyl]-1H-pyrazol-4-yl}methylidene] amino}-5-oxo-2-thioxoimidazoli-din-4-ylidene)ethanoate (4c).
IR (KBr, v_{max} cm^{-1}): 3121 (N–H-str), 3033, 2950 (C–H-str), 1711 (C=O ester), 1636 (C=O cyclic amide), 1598 (C=N), 1096 (C=S), 1240, 1188 (C–O ester); ^{1}H NMR (400 MHz, DMSO-d_6): δ 2.55 (s, 3H, SCH$_3$), 3.79 (s, 3H, OCH$_3$), 6.65 (s, 1H, C=CH), 7.40–7.77 (m, 4H, Ar-H), 8.48 (s, 1H, pyrazole-5H), 8.64 (s, 1H, N=CH), 12.75 (s, 1H, pyrazole–NH), 13.48 (s, 1H, imidazole–NH); MS: m/z = 402.0 (M + 1); Anal. calcd. for $C_{17}H_{15}N_5O_3S_2$: C, 50.81; H, 3.74; N, 17.44; Found: C, 50.79; H, 3.71; N, 17.41%.

2.3.1.4. Methyl(2Z)-[3-({(E)-[3-(4-methylphenyl)-1H-pyrazol-4-yl]methylidene}amino)-5-oxo-2-thioxoimidazolidin-4-ylidene]ethanoate (4d).
IR (KBr, v_{max} cm^{-1}): 3241 (N–H-str), 3029, 2950 (C–H-str), 1706 (C=O ester), 1641 (C=O cyclic amide), 1611 (C=N), 1098 (C=S), 1240, 1195 (C–O ester); ^{1}H NMR (400 MHz, DMSO-d_6): δ 2.38 (s, 3H, CH$_3$), 3.80 (s, 3H, OCH$_3$), 6.65 (s, 1H, C=CH), 7.35–7.65 (m, 4H, Ar-H), 7.96 (s, 1H, pyrazole-5H), 8.42 (s, 1H, N=CH), 12.72 (s, 1H, pyrazole–NH), 13.41 (s, 1H, imidazole–NH); MS: m/z = 370.1 (M + 1); Anal. calcd. for $C_{17}H_{15}N_5O_3S$: C, 55.23; H, 4.06; N, 18.95; Found: C, 55.21; H, 4.05; N, 18.93% (see Table 1).

2.4. Antibacterial studies

The antibacterial activity of newly synthesized compounds 2a–d and 4a–j was determined by well plate method (Arthington-Skaggs et al., 2000) in Mueller–Hinton Agar. The in vitro antibacterial activity was carried out against 24 h old cultures of bacterial strains. In this work, Escherichia coli, Bacillus subtilis, Salmonella typhimorium, Clostridium profingens and Pseudomonas aeruginosa were used to investigate the activity. The test compounds were dissolved in dimethyl sulfoxide (DMSO) at concentration of 1 and 0.5 mg/mL. Twenty milliliters of sterilized agar media was poured into each pre-sterilized Petri dish. Excess of suspension

Table 1 Characterization data of the compounds 2a,b and 4a–d.

| Compound	Ar	Molecular formula (Mol. wt.)	Yield (%)	M.p. (°C)
2a	2,4-Dichlorophenyl	$C_{24}H_{16}Cl_2N_4$ (431.3)	72	210–212
2b	4-Anisyl	$C_{25}H_{20}N_4$ (376.4)	68	180–182
4a	2,4-Dichlorophenyl	$C_{16}H_{11}Cl_2N_5O_3S$ (424.2)	81	282–284
4b	2,5-Dichlorothiophene	$C_{14}H_9Cl_2N_5O_3S_2$ (430.2)	86	280–282
4c	4-Thioanisyl	$C_{17}H_{15}N_5O_3S_2$ (401.4)	84	230–232
4d	4-Tolyl	$C_{17}H_{15}N_5O_3S$ (369.3)	80	286–288

Table 2 Antibacterial activity of the compounds **2a,b** and **4a–d**.

Compound	Bacillus subtilis		Escherichia coli		Clostridium profingens		Salmonella typhimorium		Pseudomonas aureginosa	
Concn (μg/mL)	1000	500	1000	500	1000	500	1000	500	1000	500
2a	6 ± 0.01	5 ± 0.01	10 ± 0.03	9 ± 0.02	6 ± 0.02	5 ± 0.02	3 ± 0.02	2 ± 0.01	4 ± 0.01	3 ± 0.02
2b	8 ± 0.01	7 ± 0.02	14 ± 0.02	8 ± 0.01	6 ± 0.01	5 ± 0.02	6 ± 0.02	5 ± 0.01	6 ± 0.01	4 ± 0.01
4a	8 ± 0.02	7 ± 0.02	8 ± 0.01	7 ± 0.02	7 ± 0.03	6 ± 0.03	4 ± 0.02	3 ± 0.01	7 ± 0.01	6 ± 0.03
4b	8 ± 0.01	7 ± 0.01	9 ± 0.01	8 ± 0.03	12 ± 0.01	11 ± 0.02	10 ± 0.01	9 ± 0.01	9 ± 0.03	8 ± 0.02
4c	10 ± 0.02	9 ± 0.02	12 ± 0.02	9 ± 0.02	16 ± 0.01	15 ± 0.02	12 ± 0.01	11 ± 0.02	16 ± 0.02	15 ± 0.02
4d	9 ± 0.01	8 ± 0.01	5 ± 0.01	4 ± 0.01	6 ± 0.01	5 ± 0.01	6 ± 0.01	5 ± 0.02	4 ± 0.01	3 ± 0.01
Standard Streptomycin	21 ± 0.02	11 ± 0.02	16 ± 0.02	10 ± 0.01	17 ± 0.02	16 ± 0.01	18 ± 0.02	17 ± 0.01	13 ± 0.02	9 ± 0.01

was decanted and plates were dried by placing in an incubator at 37 °C for an hour. About 60 μL of 24 h old culture suspension was poured and neatly swabbed with the pre-sterilized cotton swabs. Six millimeter diameter well was then punched carefully using a sterile cork borer and 30 μL of test solutions of different concentrations were added into each labeled well. The plates were incubated for 24 h at 37 °C. The inhibition zone that appeared after 24 h, around the well in each plate were measured as zone of inhibition in mm. Experiments were triplicates and standard deviation was calculated. The antibacterial results were compared with streptomycin and summarized in Table 2.

2.5. In silico molecular docking studies

The ligands were drawn in ChemDraw Ultra 6.0 (ChemOffice package) assigned with proper 2D orientation and the structure of each compound was analyzed for connection error in bond order. OSIRIS, an ADMET based Java library layer that provides reusable cheminformatics functionality which is an entirely in-house developed drug discovery informatics system was used to predict the total drug score via in silico (Sander et al., 2009). Energy of the molecules was minimized using Dundee PRODRG2 server (Schuttelkopf and Aalten, 2004). The energy minimized compounds were then read as input for AutoDock 4.2, in order to carry out the docking simulation (Morris et al., 1998). All the heteroatoms were removed from the2VF5.pdb, to make complex receptor free of any ligand before docking. The Graphical User Interface program "AutoDock Tools" was used to prepare, run, and analyze the docking simulations. Kollman united atom charges, solvation parameters and polar hydrogen's were added to the receptor for the preparation of protein in docking simulation. Since ligands are not peptides, Gasteiger charge was assigned and then non-polar hydrogens were merged. AutoDock requires pre-calculated grid maps, one for each atom type, present in the ligand being docked as it stores the potential energy arising
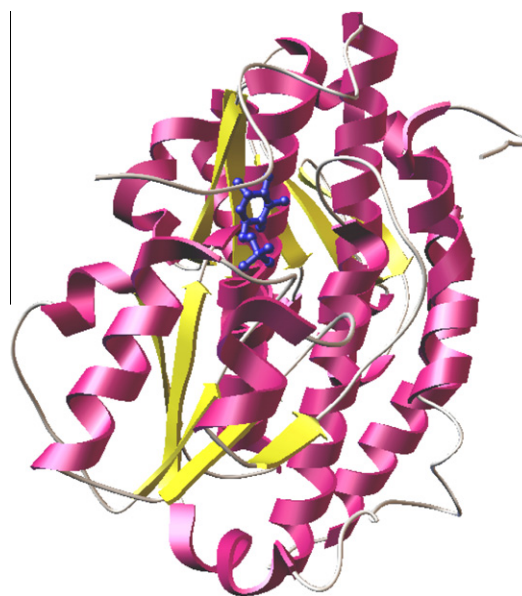
Figure 1 Crystal structure of X chain of GlcN-6-P synthase in complex with glucosamine-6-phosphate.
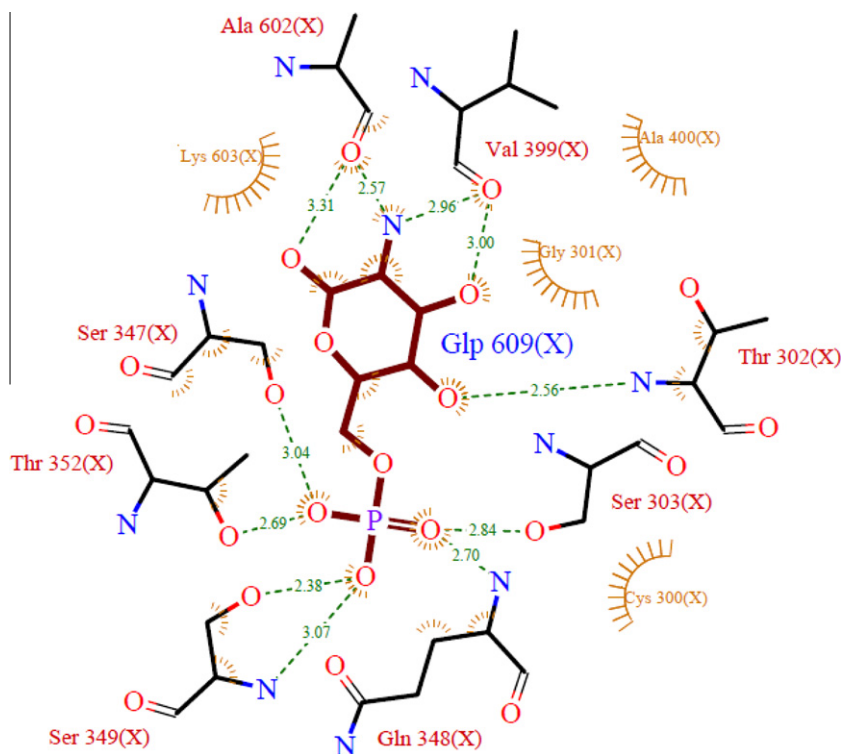
Figure 2 PDBsum's ligplot results for 2VF5, showing all 12 amino acid residues of active pocket.

from the interaction with macromolecule. This grid must surround the region of interest (active site) in the macromolecule. In the present study, the binding site was selected based on the amino acid residues, which are involved in binding with glucosamine-6-phosphate of GlcN-6-P synthase as obtained from PDB with ID 2VF5 which would be considered as the best accurate active region as it is solved by experimental crystallographic data (Mouilleron et al., 2008). Therefore, the grid was centered at the region including all the 12 amino acid residues (Ala602, Val399, Ala400, Gly301, Thr302, Ser303, Cys300, Gln348, Ser349, Thr352, Ser347 and Lys603) that surround active site as in Fig. 2. The grid box size was set at 70, 64, and 56 Å for x, y and z respectively, and the grid center was set to 30.59, 15.822 and -3.497 for x, y and z respectively, which covered all the 12 amino acid residues in the considered active pocket. Docking software AutoDock 4.2 Program supplied with AutoGrid 4.0 and AutoDock 4.0 was used to produce grid maps. The spacing between grid points was 0.375 angstroms. The Lamarckian Genetic Algorithm (LGA) was chosen to search for the best conformers. During the docking process, a maximum of 10 conformers was considered for each compound. All the AutoDock docking runs were performed in Intel Centrino Core2Duo CPU @ 2.20 GHz of IBM system origin, with 2 GB DDR2 RAM. AutoDock 4.0 was compiled and run under Microsoft Windows XP operating system.

3. Results and discussion

3.1. Synthesis of imidazole derivatives and characterization

3-Substituted-1*H*-pyrazole-4-carbaldehydes (**1a–d**) were synthesized by the Vilsmayer Haack reaction of semicarbazones

(Lebedev et al., 2005). 2,4,5-Trisubstituted imidazoles (**2a–b**) were obtained in excellent yields by refluxing 3-substituted-1*H*-pyrazole-4-carbaldehydes with 1,2-diketone (Benzil) and ammonium acetate in acetic acid for 6–7 h via Debus reaction (Vijesh et al., 2011). The imidazolones (**4a–d**) were obtained in good yield by refluxing substituted thiosemicarbazones (**3a–d**) with dimethylacetylenedicarboxylate (DMAD) in methanol for 1 h (Vijesh et al., 2011). The starting material **3a–d** in turn was synthesized by refluxing equimolar amount of 3-aryl-1-*H*-pyrazole-4-carbaldehyde with thiosemicarbazide in the presence of anhydrous sodium acetate in ethanol. The reaction pathway has been summarized in Scheme 1. Newly synthesized compounds (**2a,b** and **4a–d**) were characterized by IR, NMR, mass spectral and C, H, N elemental analyses.

Formation of 4-(4,5-aryl-1*H*-imidazol-2-yl)-3-[substituted]-1*H*-pyrazole (**2a,b**) and methyl (2*Z*)-[3-({(E)-[3-(substituted)-1*H*-pyrazol-4-yl]methylidene}amino)-5-oxo-2-thioxoimidazolidin-4-ylidene]ethanoate (**4a–d**) was confirmed by recording their IR, ^{1}H NMR, ^{13}C NMR and mass spectra. All compounds were characterized after recrystallization from appropriate solvents. IR spectrum of compound **4c** showed absorption at 3121 cm^{-1} which is due to the NH stretching. Bands at 1711, 1636 cm^{-1} are due to C=O of ester and cyclic amide respectively. Band at 1598 cm^{-1} is due to C=N, band at 1096 cm^{-1} is due to C=S group. The C–O stretching frequency of ester appeared at 1240 cm^{-1} and 1188 cm^{-1} further confirms the structure. The ^{1}H NMR spectrum of **4c** showed a singlet at δ 2.55 is due to SCH$_3$ protons. A singlet at δ 3.79 is due to OCH$_3$ protons. A singlet at δ 6.65 is due to C=CH. Aromatic protons appeared as multiplet at δ 7.40–7.77. Pyrazole-5H appeared as a singlet at δ 8.48. Similarly a singlet appeared at δ 8.64 is due to –N=CH protons. Two singlets at δ 12.75 and δ 13.48 are due to pyrazole–
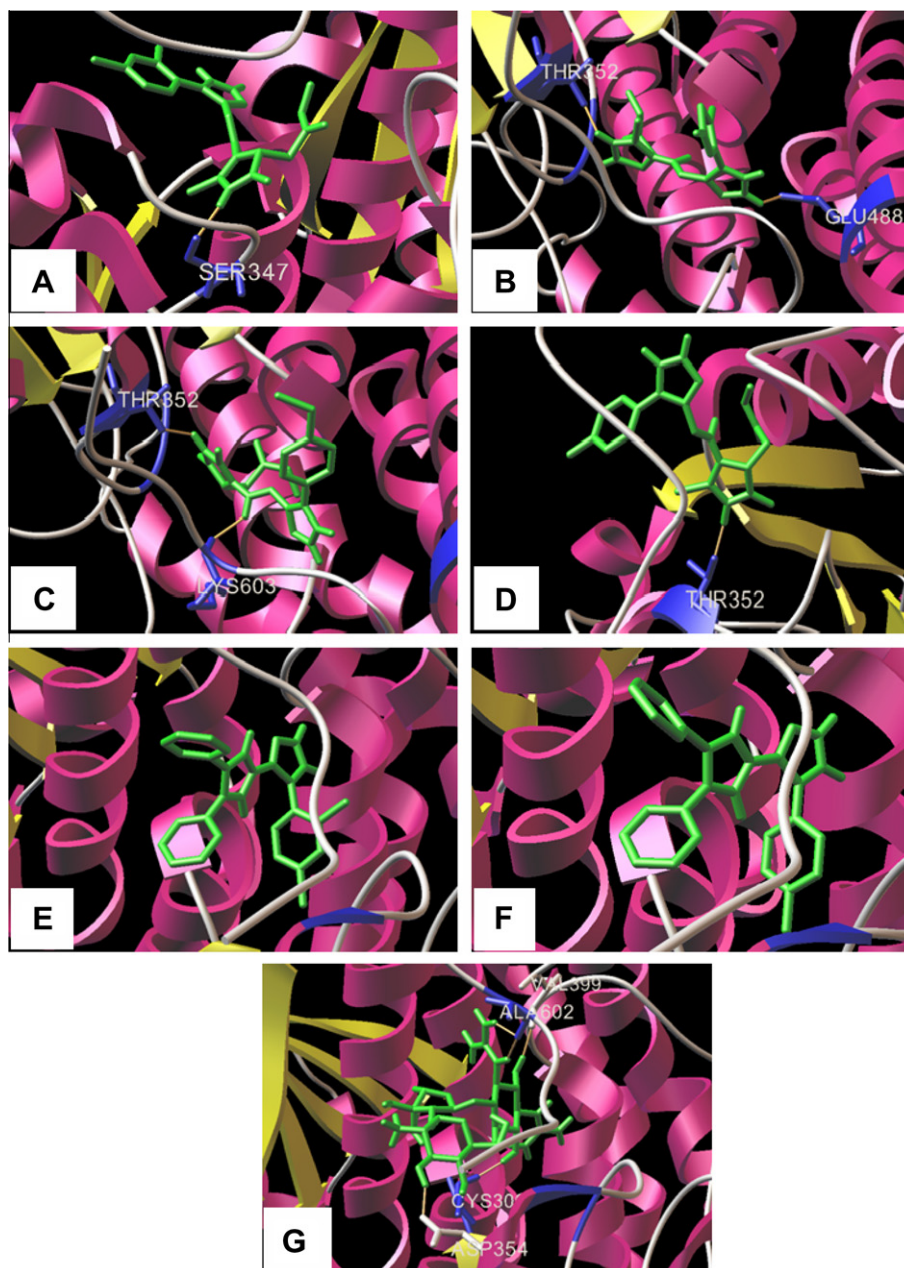
Figure 3 The best and stable conformations of all the synthesized molecules including the considered standard drug molecule. (A) **4a** forming 1H bond with Ser347. (B) **4b** forming 2H bond with Thr352 and Glu488. (C) **4c** forming 2H bond with Thr352 and Lys603. (D) **4d** forming 1H bond with Thr352. (E) **2a** forming 0H bond. (F) **2b** forming 0H bond. (G) Streptomycin forming 5H bond with Val399, Cys300, Asp354 and Ala602 (2H bonds).

NH and imidazole–NH respectively further confirms the structure. The mass spectrum of **4c** showed molecular ion peak at $m/z = 402.0\,(M+1)$, which is in agreement with the molecular formula $C_{17}H_{15}N_5O_3S_2$. Similarly the spectral values for all the compounds and C, H, N analyses are given in the experimental part and the characterization is provided in Table 1.

3.2. Antibacterial studies

The *in vitro* antibacterial activity of newly synthesized compounds **2a,b** and **4a-d** was determined by well plate method. The antibacterial screening revealed that some of the tested compounds showed good inhibition against various tested microbial strains. The result indicated that among the tested compounds, **4c** showed excellent activity against *P. aeruginosa* at concentrations of 1 and 0.5 mg/mL compared to standard drug streptomycin. **4c** showed similar activity as that of standard, against *C. profingens*, at 1 and 0.5 mg/mL concentrations. Results of antibacterial studies have been presented in Table 2.

As regards the relationships between the structure of the heterocyclic scaffold and the detected antibacterial properties, it showed varied biological activity. Compound **4c** has thioanisyl moiety on pyrazole ring, which is accounted for the enhanced

Table 3 Binding Energy and Inhibition Constant of the compounds (**2a,b**), (**4a–d**) and the standard drug Streptomycin.

Compound	Binding Energy (kJ mol^{-1})	Inhibition Constant (μM)
2a	−7.57	2.81
2b	−8.01	1.35
4a	−6.99	7.47
4b	−6.93	8.38
4c	−6.91	8.56
4d	−7.19	5.38
Streptomycin	−6.72	11.82

antibacterial activity. However in the first series, compounds showed moderate antimicrobial activity. Imidazole and pyrazole nucleus which is present in both the series are responsible for the biological activity. However the presence of other substituents is responsible for the varied biological activity of the compounds.

3.3. Molecular docking studies

Considering the well obtained *in vitro* results, it was thought worthy to perform molecular docking studies, hence screening the compounds, inculcating both *in silico* and *in vitro* results. Considering GlcN-6-P synthase as the target receptor, comparative and automated docking studies with newly synthesized candidate lead compounds was performed to determine the best *in silico* conformation. The Lamarckian genetic algorithm, inculcated in the docking program AutoDock 4.2, was employed to satisfy the purpose. Fig. 3 shows the native crystal structure of GlcN-6-P synthase (X chain) in complex with glucosamine-6-phosphate was obtained from Protein Data Bank (http://www.pdb.org/pdb/home/home.do) with the PDB ID 2VF5 which was resolved at 2.90 Å using X-ray diffraction (Mouilleron et al., 2008) (Fig. 1).

The docking of receptor GlcN-6-P with newly synthesized candidate ligands exhibited well established bonds with one or more amino acids in the receptor active pocket. The active pocket was considered to be the site where glucosamine-6-phosphate-complexes in GlcN-6-Pof 2VF5. The active pocket consisted of 12 amino acid residues as Ala602, Val399, Ala400, Gly301, Thr302, Ser303, Cys300, Gln348, Ser349, Thr352, Ser347 and Lys603 as shown in Fig. 2. The synthesized ligand molecules having 2D structure were converted to energy minimized 3D structures and were further used for *in silico* protein–ligand docking. All the six synthesized molecules were docked. Fig. 3 shows the docked images of selected candidate ligands including the considered standard drug i.e. Streptomycin. Table 3 shows the Binding Energy and Inhibition Constant of seven compounds including the standard. *In silico* studies revealed all the synthesized molecules showed good binding energy toward the target protein ranging from −8.01 to −6.91 kJ mol^{-1}.

4. Conclusion

Six new imidazole derivatives were synthesized in reasonably good yields. They were characterized by ^{1}H NMR, ^{13}C NMR, mass spectrometry, IR studies and elemental analyses. All the newly synthesized compounds were tested for antimicrobial activity by well plate method. Among the screened samples, compound **4c** has emerged as most active against all tested microorganisms compared to the standard drug.

Finally the molecular docking studies of the synthesized compounds were carried out and the results of such studies were reported. *In silico* studies revealed that all the synthesized compounds **2a,b**, **4a–d** have relatively lesser binding energy as compared to the standard drug and may be considered as a good inhibitor of GlcN-6-P. Hence this study has widened the scope of developing these imidazole derivatives as promising antibacterial agents.

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